

AMENDMENTS TO THE SPECIFICATION:

- Please add the following new paragraph on page 2, line 1 before the heading “FIELD OF THE INVENTION”:

-- CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of co-pending U.S. Patent Application Serial No. 09/755,630 filed January 5, 2001, which claims the benefit of U.S. Provisional Application Serial No. 60/174,669 filed on January 6, 2000.--

- Please delete the paragraph beginning at page 74, line 13, and replace it with the following substitute paragraph:

--Site specific mutations were introduced into patatin by first incorporating part of the a-factor signal sequence (*Pichia* expression manual, Invitrogen, Carlsbad, CA) to the patatin gene using PCR. Primers used for the PCR were GGAGCTCGAGAAAAGAGAGGCTGAAGCTCAGTTGGGAGAAATGGTGACTGTTCT (SEQ ID NO: 3) (*Xho*I site in italics) and GGTCTAGAG *GAATT*CTCATTAATAAGAAG (SEQ ID NO: 4) (*Eco*RI site in italics). The primers contained restriction sites to facilitate cloning into *Pichia pastoris* yeast secretion vector pPIC9 (GenBank accession number Z46233; Invitrogen, Carlsbad, CA). Typical PCR conditions are 25 cycles 94 °C denaturation for 1 minute, 45 °C annealing for one minute and 72 °C extension for 2 minutes; plus one cycle 72 °C extension for 10 minutes. A 50 mL reaction contained 30 pmol of each primer and 1 mg of template DNA; and 1 X PCR buffer with MgCl₂, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP, 2.5 units of *Pwo* DNA polymerase. PCR reactions are performed in RoboCycler Gradient 96 Temperature Cycler (Stratagene, La Jolla, CA).--

- Please delete the paragraph beginning at page 76, line 17, and replace it with the following substitute paragraph:

--Pat17 was expressed in *E.coli* using the pET expression system (Novagen, WI). The coding region of the mature Pat17 gene (without its signal peptide) was amplified by PCR using the primers 5'-GGGCCATGGCGCAGTTGGGAGAAATGGTG-3' (SEQ ID NO: 294) (*NcoI* site in italics) and 5'-AACAAAGCTTCTTATTGAGGTGCGGCCGCTTGCATGC-3' (SEQ ID NO: 295) (*NotI* site in italics) using standard PCR reaction conditions as described in the Gene Amp kit (Perkin-Elmer Cetus, CT) and an annealing temperature of 40°C. The template was plasmid pMON26820. The resulting DNA was digested with *NcoI* and *NotI* and cloned into a modified pET24d plasmid, designed to add an N-terminal hexa-histidine tag to the protein. The correct sequence of the PCR product was verified by sequencing, and the plasmid was transformed into *E.coli* BL21 (DE3), and transformants selected on LB containing 25 mg/mL kanamycin. The expression strain was grown in LB containing 25 mg/mL kanamycin and induced for 8 hrs at 28 °C with 1 mM IPTG. Cells were harvested and washed in 50 mM Tris/HCl pH 8.5, 150 mM NaCl, and lysed by French Press at 20,000 psi. His-tagged protein was recovered in the soluble fraction of lysed cells and subsequently purified using Ni-NTA resin as described in the QIAexpressionist manual (Qiagen CA). The partially purified protein was then dialyzed against 25 mM Tris/HCl pH 7.5 (buffer A) and loaded onto Mono Q HR 10/10 anion-exchange column (Amersham Pharmacia, NJ) equilibrated with buffer A. The protein was eluted with 25 mM Tris/HCl pH 7.5, 1 M KCl (buffer B) using a linear gradient of 0-100% buffer B run over 30 min at a flow rate of 4 mL/min using an HPLC system (Shimadzu). Fractions containing protein were assayed for esterase activity. Esterase active fractions were pooled, concentrated and dialyzed against 25 mM Tris/HCl pH 7.5 and stored at 4 °C.--

- At pages 87-174 of the Specification, please delete the Sequence Listing.
- At page 87, please insert the enclosed substitute Sequence Listing (96 pages).